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Kinetic Analysis of the Peritoneal Transport of Quinolonecarboxylic Acids in Rats

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In order to describe quantitatively the peritoneal transport of quinolonecarboxylic acids (quinolones), we employed a kinetic model based on the hydrodynamic pore theory of transcapillary exchange, which involves the changes in the volume and osmolality of the dialysate as well as the diffusion and convection of drugs across the peritoneum. Among the quinolones, we tested cinoxacin, enoxacin, norfloxacin, ofloxacin and a new quinolone, NY-198. The observed data on the osmolality, volume and drug concentration in the dialysate (hypertonic and hypotonic) after intraperitoneal administration were analyzed according to the kinetic model to estimate the hydrodynamic parameters of peritoneal transport, *i.e.*, apparent capillary membrane permeability and the reflection coefficient, of each drug by a computer-aided curve-fitting procedure. It was found that the peritoneal transport of the quinolones examined was diffusion-limited and was not affected by the tonicity of the dialysate, indicating that solvent drag played a minor role in the peritoneal transport of these drugs.

Keywords——quinolonecarboxylic acid; quinolone; peritoneal transport; peritoneal dialysis; pore theory; solvent drag; capillary membrane permeability; reflection coefficient

Quinolonecarboxylic acid derivatives (quinolones) have been used primarily in the treatment of urinary infections by the oral route of administration. These antimicrobial agents are candidates for the treatment of bacterial peritonitis caused by undergoing continuous ambulatory peritoneal dialysis (CAPD), which is widely used as an alternative to hemodialysis in patients with end-stage renal disease[1] and is also used in targeted drug delivery to tumors within the peritoneal cavity.[2] Recently, Flessner *et al.*[3] developed a distributed model of peritoneal-plasma transport of hydrophilic compounds and macromolecules, whereas Janicke *et al.*[4] proposed a comprehensive modeling approach in terms of the conventional pharmacokinetic clearance concept. In the previous studies, however, an explicit approach to the changes in the dialysate volume was not made except for the approximation presented by an exponential profile,[5] although the dialysate volume is one of the factors which directly determines the drug concentration in the peritoneal cavity. In a recent report,[6] we presented a kinetic model of peritoneal drug transport based on the hydrodynamic pore theory of transcapillary exchange,[7] in which the changes in dialysate volume and osmolality were incorporated. In the present study, this model was applied to the peritoneal transport of quinolones, *i.e.*, cinoxacin, enoxacin, norfloxacin, ofloxacin and a newly developed difluorinated quinolone, NY-198[8] (Fig. 1). Furthermore, we examined the relationship between the apparent capillary membrane permeability and the lipophilicity among the quinolones examined, to gain an insight into the mechanism of the peritoneal transport of these drugs.

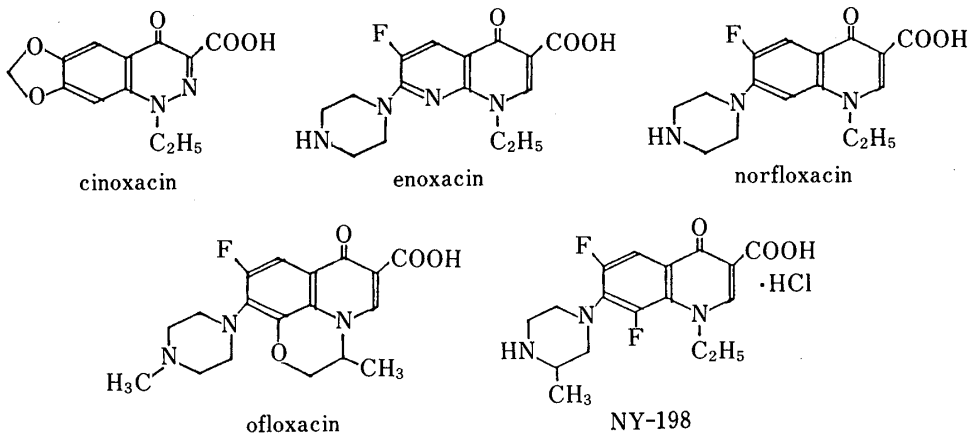

Fig. 1. Chemical Structures of Quinolonecarboxylic Acid Derivatives

Experimental

Materials——Blue dextran (BD) with an average molecular weight of 2000000 was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ofloxacin was kindly supplied by Daiichi Seiyaku Co., Ltd. (Tokyo, Japan) and cinoxacin by Shionogi & Co., Ltd. (Osaka, Japan). Enoxacin, norfloxacin and NY-198 were synthesized in the Central Research Laboratory, Hokuriku Seiyaku Co., Ltd. (Fukui, Japan). The purities of these quinolones were found to be more than 99.5% by high-performance liquid chromatography (HPLC). Other chemicals were of analytical grade and were used without further purication.

Surgical Procedure——Adult male Wistar rats (Nihon Clea, Tokyo, Japan) weighing 230—270 g were used throughout the experiments. The rats were anesthetized with an intramuscular injection of sodium pentobarbital (50 mg/kg) 30 min prior to surgery. The operating procedure employed was essentially the same as the method of Flessner *et al.*[3] Briefly, polyethylene catheters were inserted into the peritoneal cavity (multiholed tubing, 3 mm i.d.) for instillation and sampling of the peritoneal fluid and into the femoral artery (PE-50 tubing) for blood sampling. Body temperature was maintained at 37 ± 0.4 °C with heating lamps.

Peritoneal Transport Experiments——Transport experiments were performed using Krebs–Ringer solution containing 0.2% (w/v) BD and 100 μg/ml (or 13.2 mg/kg) of drug and adjusted with NaCl to 1.8 (hypertonic) or 0.45 (hypotonic) times NaCl equivalent values. The respective osmolalities in the dialysate used were measured with an osmometer (model 3D-II, Advance Instrument, MA, U.S.A.) to be 0.55 and 0.15 osm/kg. Thirty-three milliliters of Krebs–Ringer solution (pH 7.4, 37°C) containing 0.2% (w/v) BD (as a volume marker) and drug (100 μg/ml) was introduced into the peritoneal cavity through the implanted catheter. At designated times after initiation of dialysis, peritoneal samples (0.6 ml) were collected in a 1.0 ml syringe by aspiration through the catheter. Blood samples (0.2 ml) were withdrawn from the femoral artery at specific time intervals. The volume, osmolality and drug concentration in the dialysate were simultaneously determined.

Measurement of Oil–Water Partition of Quinolones——In order to examine the relationship between the lipophilicity and the peritoneal capillary membrane permeability, the apparant partition coefficient (P_{app}) of each quinolone tested was measured at 37°C using *n*-octanol/50 mM sodium phosphate buffer (pH 7.0, ionic strength 0.15) according to the method of Tsuji *et al.*[9]

Analytical Methods——The determinations of the dialysate volume and the osmolality in the plasma and dialysate samples were described in a previous report.[6] For the determination of the quinolones, a HPLC (Shimadzu LC-6A) was equipped with either an ultraviolet detector (Shimadzu SPD-6A) to assay cinoxacin, enoxacin, norfloxacin and ofloxacin (at 254 nm) or a spectrofluorometer (FP110, Japan Spectroscopic Co., Ltd., Tokyo, Japan) to assay NY-198 (excitation at 340 nm, emission at 460 nm). Cosmosil 5-C_{18} (4.6 × 150 mm, Nakarai Chemicals Ltd., Kyoto, Japan) was used as a stationary phase. The HPLC conditions used were as follows: mobile phase, acetonitrile–0.05 M citric acid–1 M ammonium acetate (15 : 84 : 1, v/v); flow rate, 1.0 ml/min; sample volume, 20 μl. The retention times of cinoxacin, enoxacin, norfloxacin, ofloxacin and NY-198 were 7.2, 8.5, 10, 9.3 and 10 min, respectively.

Data Analysis——In the previous report,[6] we presented a kinetic model to describe the peritoneal transport of solvent, osmotic solute (sodium chloride) and drug according to an equivalent pore theory of transcapillary exchange. Concerning the changes in the volume (V^D), osmolality (C^D_{osm}) and drug concentration (C^D_d) in the dialysate after intraperitoneal administration, the differential equations in the final forms can be written as follows:

$$\frac{dV^D}{dt} = -J_v = -J_0 + L_p S \cdot RT\sigma_s \Delta C_{osm} \quad (1)$$

$$\frac{dC^{D}_{osm}}{dt}=[J_v\{(1+\sigma_s)C^{D}_{osm}-(1-\sigma_s)C^{P}_{osm}\}/2-P_s\Delta C_{osm}]/V^{D} \quad (2)$$

$$\frac{dC^{D}_{d}}{dt}=\{J_v(1+\sigma_d)/2-P_d\}C^{D}_{d}/V^{D} \quad (3)$$

where J_v and L_pS represent the net volume flux (ml/min) and hydraulic conductivity (ml/min/atm) across the peritoneum; RT is the product of the gas constant (atm·ml/K/mol) and the temperature in degrees Kelvin (K); C^{P}_{osm} represents the osmolality (osm/kg) in plasma; P_s and σ_s represent the apparent capillary membrane permeability and the reflection coefficient of sodium chloride, respectively; P_d and σ_d are the apparent capillary membrane permeability and the reflection coefficient of drug, respectively; and J_0 is a hybrid term expressed as:

$$J_0=L_pS(\Delta P-\sigma_{prot}\Delta\Pi_{prot})-L \quad (4)$$

where ΔP is the transperitoneal hydrostatic pressure difference (atm); σ_{prot} and $\Delta\Pi_{prot}$ are the reflection coefficient and transperitoneal oncotic pressure difference (atm) of the total proteins, respectively; and L is the net lymph flow rate (ml/min) from the peritoneal cavity.

Since P_d may be affected not only by the intrinsic permeability across the capillary membrane but also by the peritoneal effective blood flow and diffusion through the unstirred water layer adjacent to the capillary membrane, P_d is an "apparent" or "functional" parameter.

In order to estimate the hydrodynamic parameters (L_pS, P_s and J_0) for the solvent and solute (NaCl), the obtained data on dialysate volume and osmolality were fitted to the numerical solution of the simultaneous differential equations (Eqs. 1 and 2) by a nonlinear least-squares regression analysis (NONLIN74[10]) using a digital computer (FACOM-M360AP) at the Information Processing Center, Kanazawa University. The values of R and T were taken to be 0.082 atm 1/K/mol and 310 K, respectively. Subsequently, the hydrodynamic parameters for the drugs (P_d and σ_d) were obtained by simultaneously fitting the profiles of the volume, osmolality and drug concentration in the dialysate using Eqs. 1, 2 and 3 at the fixed values of J_0, L_pS, P_s and σ_s.

Results and Discussion

Figures 2A and 2B present the time courses of the volume and osmolality of the dialysate (containing ofloxacin) under hypertonic and hypotonic conditions, respectively. As can be clearly seen from the figures, the observed data corresponded well with the simulation curves using the reported values of the hydrodynamic parameters for solvent and an osmotic solute (NaCl),[6] *i.e.*, L_pS (4.69 ml/min/atm), P_s (0.728 ml/min), σ_s (0.01), and J_0 (0.0183 ml/min). Moreover, these changes were identical to those observed in the presence of other quinolones or in the absence of any quinolone in the dialysate, indicating that the drug concentrations in the dialysate were so low that the drug did not contribute significantly to the net volume flux (J_v) through the peritoneum. Since the osmolality in plasma (C^{P}_{osm}) was determined to be almost 0.30 osm/kg and was unchanged throughout the transport experiments, C^{P}_{osm} was regarded as a constant and not a variable parameter. Using the reported values of unbound fraction (f_p) with rat plasma proteins[11,12] (<0.1, 0.655, 0.857, 0.772, 0.719 for cinoxacin, enoxacin, norfloxacin, ofloxacin and NY-198, respectively), the plasma unbound concentrations ($f_pC^{P}_{d}$) of each quinolone were determined to be less than a tenth of the dialysate concentrations (C^{D}_{d}) during the peritoneal dialysis (results not shown), so that the assumption of $f_pC^{P}_{d}\ll C^{D}_{d}$ in the derivation of the differential equations (Eqs. 1—3) seems to be valid.

Figures 3A—E present the peritoneal disappearance curves of the five quinolones under hypertonic and hypotonic conditions, respectively. The semilogarithmic plots showed two different curvatures; concave and convex for the hypotonic and hypertonic dialysates, respectively. Using the same data, the disappearance curves of the total amount ($V^{D}C^{D}_{d}$) of these quinolones were similar to each other between the hypertonic and hypotonic dialysates, indicating that solvent drag (a flux coupled to water flow) played a minor role in the peritoneal transport of these drugs.

The dialysate concentration-time profiles of quinolones (Figs. 2 and 3) were fitted to Eqs. 1, 2 and 3 using the computer program NONLIN74, and the estimated hydrodynamic

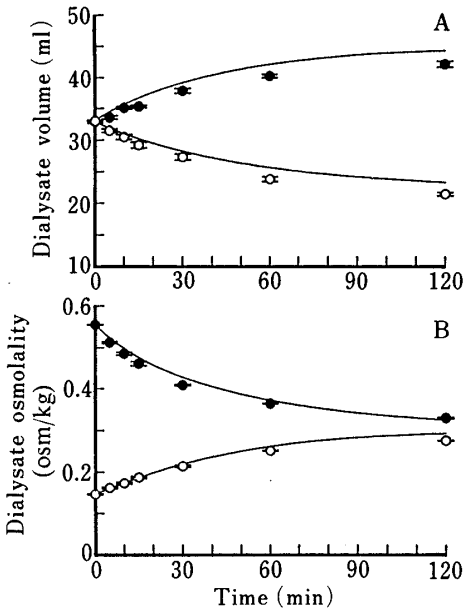

Fig. 2. Time Courses of the Dialysate Volume and Osmolality after Intraperitoneal Administration of Ofloxacin with the Hypertonic and Hypotonic Dialysates in Rats

A, Volume change; B, osmolality change. ●, Hypertonic dialysate; ○, hypotonic dialysate. Each point and vertical bar represent the mean ± S.E.M. ($n=4$).

Solid lines show the simulation curves using the hydrodynamic parameters for solvent and an osmotic solute, obtained in the previous study.[6]

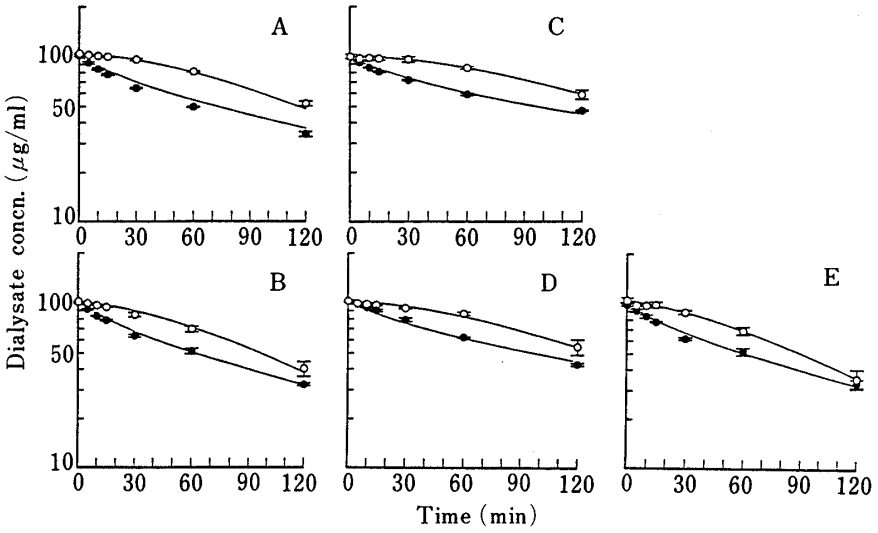

Fig. 3. Disappearance Curves of the Quinolonecarboxylic Acids from the Hypertonic and Hypotonic Dialysates in Rats

A, Cinoxacin; B, enoxacin; C, norfloxacin; D, ofloxacin; E, NY-198. ●, Hypertonic; ○, hypotonic.

Each point and vertical bar represent the mean ± S.E.M. ($n=4$). Solid lines show the simulation curves using the hydrodynamic parameters (listed in Table I) estimated by a nonlinear least-squares regression analysis.

parameters (P_d and σ_d) for the five quinolones are listed in Table I. The estimated reflection coefficient of a drug (σ_d) should represent a weighted mean value for all transport pathways across the peritoneum. As previously described,[7a,13] the σ_d value of a neutral substance is significantly related to the molecular radius, which can be expressed as a function of the molecular weight. Considering the small values of σ_d reported for water-soluble and neutral substances such as inulin (0.38) and sucrose (0.06) in rats,[3c] the σ_d values (ranging from 0.63 to 1.00) of these quinolones are relatively too large for their molecular weights (ranging from 262 to 360). The large σ_d values of the quinolones should probably be attributed to diverse molecular configuration and ionization potential. On the other hand, the P_d values of the quinolones were calculated to be 0.184—0.294 ml/min in a 250-g rat and much smaller than the effective peritoneal blood flow reported in rats (3—4 ml/min),[14] indicating that the

TABLE I. Apparent Capillary Membrane Permeability (P_d) and Reflection Coefficient (σ_d) for the Peritoneal Transport of Quinolonecarboxylic Acids in 250-g Rats[a)]

Drugs	P_d[b)] (ml/min)	σ_d[b)]
Cinoxacin	0.245±0.005	1.00±0.10
Enoxacin	0.289±0.004	1.00±0.08
Norfloxacin	0.184±0.003	0.818±0.054
Ofloxacin	0.202±0.003	0.676±0.066
NY-198	0.294±0.003	0.631±0.068

a) Each drug (13.2 mg/kg) was intraperitoneally administered, and the parameters were determined by a nonlinear least-squares regression analysis. *b*) Each value represents the mean±S.D. of the estimated parameter.

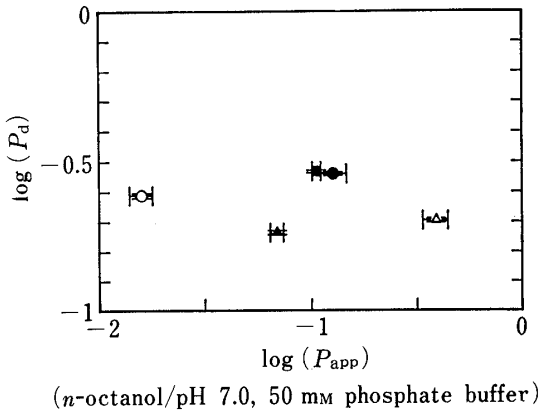

Fig. 4. Relationship between the Apparent Partition Coefficient (P_{app}) and the Peritoneal Capillary Permeability (P_d) of Quinolonecarboxylic Acids

○, Cinoxacin; ●, enoxacin; ▲, norfloxacin; △, ofloxacin; ■, NY-198. The apparent partition coefficients were measured at 37 °C in *n*-octanol/50 mM phosphate buffer (pH 7.0). Each point and vertical bar represent the mean±S.D. of the estimated P_d (listed in Table I). Each horizontal bar represents the S.E.M. of four determinations of P_{app}.

peritoneal transport of the quinolones was diffusion-limited.

Figure 4 illustrates the relationship between the apparent peritoneal capillary membrane permeability and the lipophilicity among the quinolones examined. In general, provided that a series of drugs is transported only by passive diffusion through the lipoidal membranes of a certain tissue, a linear relationship between the permeability and lipophilicity (such as the oil–water partition coefficient) of the drugs is expected to be observed. However, there was no significant correlation between these two properties of the quinolones, indicating that the lipophilicity was not a determinant factor of the peritoneal capillary permeability of the quinolones examined. There are two possible reasons for this. First, the major route of diffusion of these drugs across the peritoneum may be the pore system through the peritoneal capillary membrane, which is not significantly affected by the intercellular water movement. Second, the diffusion through the unstirred water layer may be the limiting process in the peritoneal transport of these drugs. Since the quinolones could rapidly distribute into various tissues after intravenous injection in rats *in vivo* and into rat blood cells *in vitro*, and the tissue-to-plasma (or tissue-to-medium) concentration ratio was close to unity,[12)] it is clear that these drugs can rapidly diffuse through the membranes of various tissues. From this point of view, the tissue distribution of the quinolones could be considered to be blood flow-limited, while the present study revealed that the peritoneal transport of these drugs was diffusion-limited. The effect of the unstirred water layer on the transperitoneal exchange of the quinolones could not be examined in the present *in vivo* experiment which, however, mimics the clinical situation during peritoneal dialysis. Moreover, in contrast to certain membranes such as the brush border membranes of the small intestine and kidney, it is not known whether any electrolytes are transported *via* a carrier-mediated system across the peritoneum. In any case, further investigation is needed to clarify the mechanism of the

peritoneal transport of quinolones.

In conclusion, the kinetic model based on the hydrodynamic pore theory of transcapillary exchange could be adequately applied to the peritoneal transport of quinolones in rats. It was found that the peritoneal transport of the quinolones was diffusion-limited and that the solvent drag effect was of small importance in the peritoneal transport of these drugs. Moreover, the following two possible mechanisms were suggested concerning the peritoneal transport of the quinolones: 1) the diffusion across the unstirred water layer may be the rate-limiting step and 2) the quinolones may diffuse through the pore system, which is not significantly affected by intercellular water movement.

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